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Acidification Effects on Macroinvertebrates and Fathead Minnows ('Pimephales promelas') in Outdoor Experimental Channels

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ACIDIFICATION EFFECTS ON MACROINVERTEBRATES AND FATHEAD MINNOWS (*PIMEPHALES PROMELAS*) IN OUTDOOR EXPERIMENTAL CHANNELS

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Abstract—Three outdoor experimental channels at the U.S. EPA Monticello (Minnesota) Ecological Research Station were employed to assess in the field acidification effects on macroinvertebrate communities and populations of fathead minnows (*Pimephales promelas*). One channel served as ambient (pH 8), the second was acidified with sulfuric acid to pH 6 and the third to pH 5 for 17 weeks. Acidification did not markedly increase toxic metal concentrations in the channels; however, there was some evidence of possible mercury mobilization. Benthic macroinvertebrate densities were lower in the acidified channels during most of the study and final diversity indices were 2.1, 1.7 and 1.2 in the ambient, pH 6 and pH 5 channels, respectively. Drift of amphipods and leeches was stimulated in the pH 5 channel early in the study. The number of insect taxa and percentages emerging from the ambient, pH 6 and pH 5 channels, respectively, were 30 and 56%, 28 and 31%, and 18 and 13%. Macroinvertebrate tolerance to the acidification was classified as follows: damselflies, isopods and leeches most tolerant; chironomids, some amphipods (*Crangonyx*) and flatworms of intermediate tolerance; and other amphipods (*Hyalella azteca*) and snails (*Physa gyrina*) most sensitive. Fathead minnow spawning and embryo production were similar in the ambient and pH 6 channels with little spawning and no eyed-embryos found in the pH 5 channel. Newly-hatched larval fish did not survive to the juvenile stage in the pH 6 channel. Continued acidification would likely have further decreased the diversity of the macroinvertebrate community, increased relative abundance of more tolerant species, and caused extinction of the minnow population.

INTRODUCTION

Acidification of surface waters resulting from increased levels of acid precipitation in the United States and other parts of the world has received recent attention from many investigators. The ecological consequences of acidification, although not fully known, include damage to both terrestrial and aquatic systems (Gorham, 1976; Glass & Loucks, 1980). Case histories documenting the impact of airborne acid industrial emissions have been reported by Beamish & Harvey (1972) in Canada, Hendrey & Wright (1976) in Norway, and Schofield (1976) in the United States and by many other investigators. Canadian studies (Beamish, 1976) have shown a progressive decline in fisheries with reproductive failure of several species in lakes with pH levels from 4.5 to 6.0. Acidification effects on invertebrates were summarized by Glass & Loucks (1980).

Several groups have recommended levels of pH needed to protect aquatic life. European Inland Fisheries Advisory Commission (1969) suggested a pH range of 5.0–9.0 would not be lethal to fish, but emphasized that no definitive pH range could be given that would not be harmful to a fishery. They concluded that as natural waters deviated from a normal pH range, a gradual deterioration of aquatic life would occur. The suggestion that a pH range of 5.0–9.0 would not harm fish is not supported by laboratory studies of Mount (1973), Craig & Baksi (1977) and Ruby *et al.* (1977). A recommended pH value above 6.5 was proposed by the National Academy of Science (1973) and the U.S. Environmental Protection Agency (1976) to provide maximal protection to aquatic life. Few studies have been conducted on the acidification effects in natural streams when not complicated with heavy metal additions from acid mine wastes. Herricks & Cairns (1974) reduced the pH of a "healthy, productive stream" from 8 to below 4 for a period of 15 min. They observed a reduction in diversity and density of the macrobenthos. Hall *et al.* (1980) acidified a small soft-water stream in New Hampshire with sulfuric acid to pH 4 for 6 months and found decreased emergence of insects, increased macroinvertebrate drift, and that brook trout moved downstream out of the acidified section in spring and used groundwater seeps as refugia during the summer. They also found elevated concentrations of some metals in the acidified stream section.

The purpose of this study was to assess in the field the impacts of acidification on freshwater macroinvertebrates and fish. Three outdoor experimental chan-

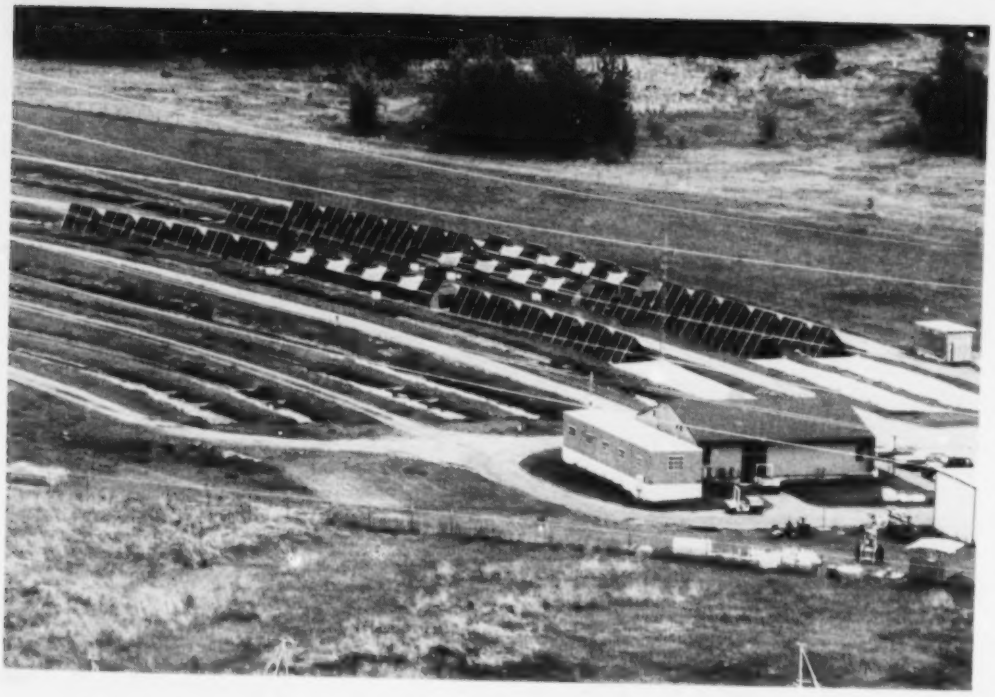

Fig. 1. View of experimental channel study sections.

nels were employed; one channel served as an ambient reference (pH 8), the second was acidified with sulfuric acid to pH 6 and the third to pH 5 for 17 weeks. The study was designed to determine if acidification would reduce a fish population, benthic invertebrate diversity and insect emergence, and increase macroinvertebrate drift. The invertebrate community in the channels was a product of natural colonization; fathead minnows (*Pimephales promelas* Rafinesque) were introduced to serve as test fish.

MATERIALS AND METHODS

Facility

The Monticello Ecological Research Station, a field station of the U.S. EPA Environmental Research Laboratory-Duluth, is adjacent to the Northern States Power Company nuclear generating plant on the Mississippi River 2 miles west of Monticello, Minnesota. The station has eight outdoor experimental channels each 520 m long with 30.5 m alternate mud-bottom pools and gravel (2–5 cm dia) riffles. The upper 122 m of three channels, including two pools and two riffles, were used in this study (Fig. 1). Water was pumped into the channels from the Mississippi River. A wier was used to raise the water level 15 cm at the downstream end of the first pool. This first pool served as a sediment trap. The two riffles in the study area were 1.4 m wide and 10–13 cm deep and the pool was 3.6 m wide and 0.6–0.7 m deep. Water flow into the three channels was maintained at 0.76 $m^3 min^{-1}$ (range 0.70–0.84). Flow velocity in the riffles was 0.1–0.2 $m s^{-1}$. A 3 mm mesh screen barrier at the end of the second riffle confined fish to the study area. A-frame wooden structures covered with black polypropylene fabric were used to reduce the light intensity (by 70–75%) and plant growth in the study area. Industrial grade (ASARCO, 66°) sulfuric acid was added continuously from 9 May to 7 September, 1979 to the upper ends of two channels. The acid was conveyed from polyethylene storage tanks (in a shed adjacent to the channel) in PVC pipes by two controlled volume Milton Roy diaphragm pumps that were equipped with back pressure valves to stabilize the pumping rate and prevent back syphoning. Acid was added to the river water as it flowed down a 2.5 m long wood trough from the wier at the upper end of the first riffle. Rate of acid addition was controlled manually to provide the desired pH. Acid additions to achieve pH 5 and pH 6, ranged from 40–62 and 27–42 ml min^{-1}, respectively. To insure thorough mixing, the trough width was tapered with deflecting baffles; the water then entered a shallow pool in the riffle gravel to insure complete mixing.

Physical and chemical methods

Measurements of pH were taken at mid-depth in the center of the first riffle of the two acidified channels and in the pools of all three channels with Leeds and Northrup 7773 pH electrodes. Electrical impulses were transmitted to receivers in the laboratory and continuously printed onto a Leeds and Northrup Speedomax W recorder. The pH receivers were calibrated weekly during the first month of the study and monthly thereafter. Mid-depth measurements of pH were made in the center of the first riffle of the ambient channel and at three depths in the pools (2, 10 and 30 cm from the bottom). Measurements were made in the morning and afternoon three times per week with a Leeds and Northrup 7417 or a Bower and Haack Model 5501C portable pH meter.

Total hardness, acidity and conductivity were measured weekly at mid-depth in the pools according to procedures

by the APHA (1975). Conductivity was measured with a Beckman Model RB4-250 meter and readings adjusted to 25°C.

Water and substrate temperatures were monitored throughout the study with type "T" thermocouples and printed on Speedomax W recorders or a Leeds and Northrup Trendscan Data 1000 Acquisition System. Thermocouples, attached to wood stakes, measured water temperature at mid-depth (30 cm from the bottom) in the pools and 1 cm above the substrate in the riffles. These thermocouples were checked for accuracy weekly during the first month of the study and monthly thereafter. Substrate temperatures were measured at the surface and 15 cm into the pool mud and 8 cm into the riffle substrate. Dissolved oxygen and total alkalinity measurements were taken at three depths in the pools (2, 10 and 30 cm from the bottom). These parameters were determined based on samples taken weekly at sunrise and 1500 CST and analyzed according to the APHA (1975).

Analyses of 12 element concentrations (Table 1) were made monthly at mid-depth in the pools. Duplicate 500 ml samples were collected with an alpha Van Dorn sampler and preserved with 2 ml concentrated nitric acid in polyethylene bottles. Duplicate samples of sulfuric acid were also collected from the storage tank. All samples were analyzed at the U.S. EPA Newtown (Ohio) Fish Toxicology Laboratory. Determinations for sodium and potassium were by flame emission, calcium by flame absorption spectrophotometry, and mercury analyses by using an automated cold vapor technique. Determinations for the remaining elements (aluminium, chromium, copper, iron, lead, manganese, nickel and zinc), were by graphite absorption procedures as prescribed by the U.S. Environmental Protection Agency (1979). Each sample was analyzed three times for each element.

Nutrient and carbon determinations were made once or twice monthly at mid-depth in the pools. Each 250 ml sample was preserved with 2 ml of a 5000 mg l^{-1} aqueous solution of mercuric chloride. The samples were analyzed at the U.S. EPA Waste Identification and Analysis Laboratory in Cincinnati, Ohio. Total organic carbon was measured with a Beckman Model 915A analyzer, Kjeldahl nitrogen with a Technicon block digester and ammonia, nitrite, nitrate nitrogen and phosphate with a Technicon autoanalyzer according to the U.S. Environmental Protection Agency (1979). Chemical oxygen demand was determined according to APHA (1975).

Macroinvertebrate procedures

Triplicate benthic samples were collected from each riffle and pool every 2 weeks from 1 May to 18 September. The first sample set was taken prior to acidification and the last set from the pools and the last two sets from the riffles were taken after acidification. Riffles were sampled with substrate-filled stainless-steel containers buried flush to the substrate surface for a period of eight weeks. The containers measured 14.3 × 12.8 × 15.0 cm deep and were modified with two 36 cm^2 screen (0.6 mm mesh) covered openings on two opposing sides. When buried, the container covered an area of 0.018 m^2. The pools were sampled with a pole-mounted Ekman grab (sampling area 0.023 m^2). Invertebrates were washed free from the substrate and concentrated in a 0.4 mm mesh sieve, then preserved in 10% formalin containing 100 mg l^{-1} rose Bengal stain.

Diel drift samples were collected monthly from 30 May to 22 August. Nets were set 5 m upstream from the lower end of the second riffle for 45 min every 2 h. Additional nocturnal (2100–0300 CST) samples were collected at irregular intervals from 26 June to 23 August. The drift nets had a mouth opening of 1350 cm^2 and a 90 cm long Nitex bag (mesh opening 350 μm). They were supported by wood frames and panels that directed all the channel water flow

Table 1. Metal concentrations in the experimental channel pools and acid storage tank*

Metal	Ambient channel				pH 6 Channel			pH 5 Channel			Acid tank		
	May	June	July	Aug.	May	June	July	Aug.	May	June	July	Aug.	June	July	Aug.
Aluminum (mg l^{-1})	0.30	0.17	0.22	0.15	0.28	0.19	0.28	0.19	0.30	0.16	0.25	0.18	0.90	0.66	0.75
Calcium (mg l^{-1})	30	37	41	43	30	38	41	43	30	38	41	43	12	13	nm†
Chromium (μg l^{-1})	0.7	0.4	0.9	0.5	0.4	0.3	1.0	0.8	0.6	0.3	1.2	0.9	610	340	1500
Copper (μg l^{-1})	3.2	2.1	3.2	2.5	1.5	3.6	2.1	2.2	4.1	2.4	2.1	2.8	3100	16000	2900
Iron (mg l^{-1})	0.71	0.49	0.75	0.35	0.67	0.61	0.84	0.46	0.72	0.54	0.80	0.44	230	280	780
Lead (μg l^{-1})	0.4	1.2	0.9	0.7	0.2	0.4	0.4	1.8	0.9	0.4	1.1	1.7	530	510	150
Manganese (μg l^{-1})	72	150	210	130	81	210	200	170	120	200	180	170	2.3	3.0	7.8
Mercury (μg l^{-1})	0.2	0.3	0.1	0.1	0.2	0.4	0.3	0.4	0.5	0.6	0.6	0.4	1600	1200	850
Nickel (μg l^{-1})	2	1	<1	<1	1	1	<1	<1	2	3	<1	<1	—	500	1000
Potassium (mg l^{-1})	2.0	2.0	1.6	1.8	2.0	2.0	1.7	1.8	2.1	2.0	1.7	1.8	3.8	3.8	20
Sodium (mg l^{-1})	3.7	4.2	3.9	5.4	3.7	4.2	3.9	5.4	3.7	4.3	4.0	5.6	96	29	65
Zinc (μg l^{-1})	5.9	5.5	6.2	2.8	8.8	5.0	4.9	2.0	17	9.2	7.2	3.0	530	650	2600

*Values are the mean of two duplicates and rounded to two significant figures.
†Not measured.

through the net. The drift samples were concentrated and preserved in the same way as the benthic samples. The benthic and drift samples were manually sorted and tabulated in the laboratory. Subsampling procedures, similar to Gerking (1962), were normally used when there were more than 200 individuals of a taxon present in the sample. Body length measurements were made of the four most abundant taxa: snails (*Physa*), isopods (*Asellus*) and amphipods (*Crangonyx* and *Hyalella*).

Statistical analyses were conducted on taxa comprising 97% of the total numbers. The non-parametric Kruskal–Wallis test was used to compare stations within the three channels. Analysis of variance was the parametric test for interchannel comparison with the control variables being channel and location. Diversity indices (Shannon & Weaver, 1949) were calculated at each sampling period for the pool and riffle benthic data in each channel.

Surface level emergence traps were located in the center of each riffle and pool. Samples were collected weekly from 17 April to 14 September. The first sample set was taken before acidification and the last set was taken after acidification. The traps and methods were described by Nordlie & Arthur (1981).

Fish procedures

On 17 April, 450 fathead minnows obtained from Crescent Lake in Sherbourne County, Minnesota were stocked in the study section of each of the three channels. Average weight and length of a random 6.7% sample of the stocked fish was 0.6 g (range 0.3–0.9 g) and 4.2 cm (range 3.2–4.7 cm), respectively. Two fish cages were placed 15 and 25 m downstream from the upper end of the pool in each channel. The cages were of wood frame construction, 1.3 × 0.9 × 0.9 m, and covered with 3.1 mm mesh polypropylene net. On 18 April, 20 fish were placed in each cage. Average weight and length of the fish were 0.7 g (range 0.3–0.9 g) and 4.3 cm (range 3.4–4.8 cm). Hereafter, the fish in cages are referred to as "caged" and those unconfined as "free-roaming".

Weekly growth measurements were recorded on the free-roaming fish during the pre-spawning period from early May to mid-June. The fish were collected with 0.8 × 0.7 × 0.5 m plexiglass framed box traps covered with 0.4 mm mesh polypropylene net. The opening at one end of the trap was a vertical slit formed by two movable plexiglass panels. Each panel was placed at a 45° angle to the length of the box. The panels extended 20 cm beyond the width and 30 cm beyond the length of the box. Traps were set overnight in the upstream, middle and downstream sections of each pool. All the trapped fish were counted and a random sample of 30–50 were anesthetized, weighed and measured. The fish were returned to the channels within 3 h. Caged fish were counted periodically and all were removed and weighed on 16 August.

Five days after free-roaming male fish began to show secondary sexual characteristics, 112 artificial spawning substrates were placed in each pool. The substrates, made of 15 × 15 × 1.5 cm untreated exterior plywood, were grouped in columns of four spaced 10 cm apart on a steel rod (Fig. 2). Four columns were suspended from each of seven wood bridges evenly spaced along the length of the pool with the uppermost substrate 5–8 cm below the water surface. Two additional columns were placed in the third pool upstream from the lower fish barrier. Eight substrate columns (26.7%) were selected at random and eggs were removed 3 times weekly (to insure removal prior to the end of the 3–4-day incubation period). Eggs were counted by water volume displacement (100 eggs equalled approx. 0.2 ml) and discarded. Egg counts were also made on one column placed in each cage. Observations on fertility and viability were made by removing the remaining substrates for no more than 20 s, 3 times per week and estimating the number of eggs and embryos present.

Longitudinal and vertical distributions of adult fish were determined during the spawning period. Box traps were placed in each pool as follows: at both the upper and lower ends and two in the middle: one at the bottom to mid-water depth and the other at mid-water to surface. Traps were also placed in the center of the first riffle to capture fish moving upstream and the center of the second riffle to capture fish swimming downstream. Pool traps were examined daily. Riffle traps were checked at 0830 and 1400 CST to determine diel longitudinal distribution patterns.

Surveys of second generation fish were conducted between 19 July and 23 August. Traps, with openings reduced to less than 1 cm, were set for 18 h periods (1700–1100 CST) in the pool of each study section and the pools downstream from the lower fish barriers. The mesh size of the downstream barrier was not small enough to prevent larval fish from moving downstream. Nocturnal import and export of fish from the study section were measured by taking 30 min drift samples at 2100, 2300 and 0000 CST weekly from 18 June to 11 July. Sampling locations were at the upper end of the first riffle and 5 m upstream from the downstream end of the second riffle.

Fathead minnows were found in the first pool (upstream from the point of acid addition) in all three channels on 24 July. The numbers removed by sein were 288 in the

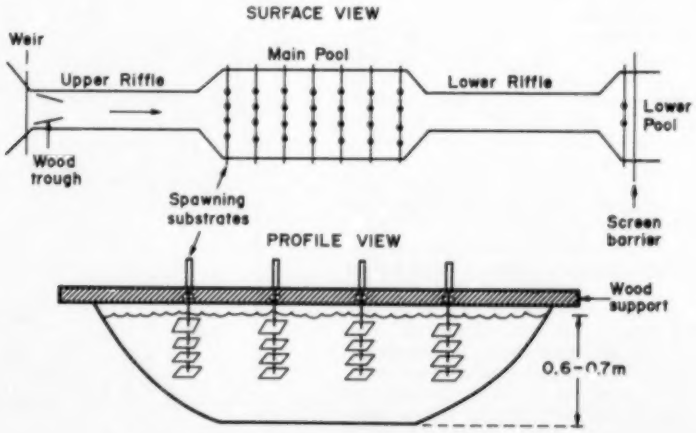

Fig. 2. Positions of fathead minnow spawning substrates.

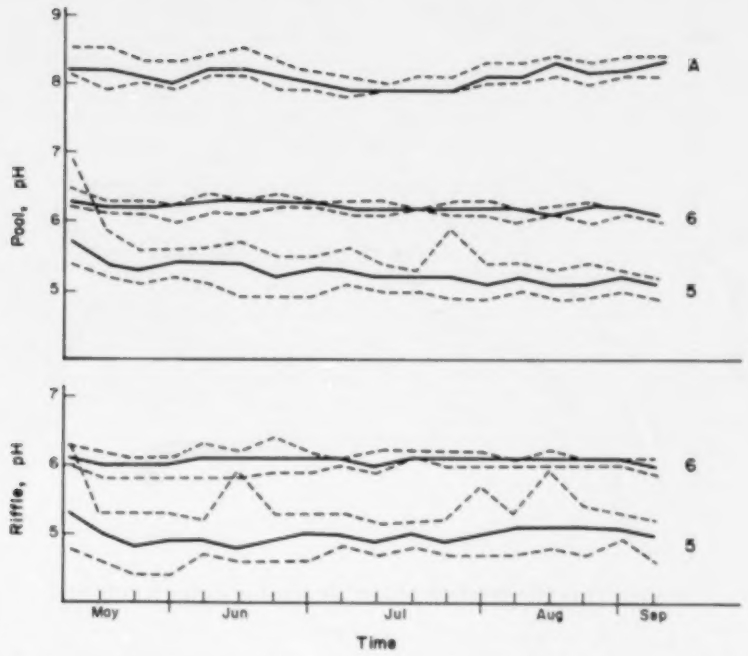

Fig. 3. Weekly mean, maximum and minimum pH in riffles and pools. Solid line is the mean and dashed lines maximum and minimum values. A = ambient channel; 6 = pH 6 channel; 5 = pH 5 channel.

ambient channel, 194 in the pH 6 channel and 69 in the pH 5 channel. To equalize the number of fish removed from the pH 6 and ambient channels, 94 were returned to the ambient channel study section. Further movement of fish out of the study area was prevented by placing a 3 mm mesh barrier 3 m downstream from the weir.

All free-roaming fish were removed on 19 September. They were counted, lengths recorded and a 10% subsample was randomly selected for weight determinations.

RESULTS

Physical and chemical comparisons

Continuous pH monitoring throughout the study revealed little intrachannel variation (Fig. 3). The ambient channel had an overall pH range of 7.8–8.5 with a range in weekly mean values (calculated according to Kinney, 1973) of 7.9–8.3. The pH 6 channel had an overall range of 5.8–6.5 with a weekly mean range of pH 6.0–6.3. The pH 5 channel had an overall range in pH of 4.4–6.9 with a weekly mean range of pH 4.8–5.7. Intrachannel differences between riffle and pool pH values were usually within 0.2 pH units.

Weekly differences between water and substrate temperatures were generally 1–3°C throughout the study (Fig. 4). Mid-water pool and substrate surface temperatures exceeded 10°C by late May and 20°C from early July to mid-August. The lowest temperature (6.5°C) occurred in early May and the highest (26.3°C) in late July. Riffle substrate temperatures were generally within 0.5°C of the mid-water pool temperatures. Temperatures at the pool substrate surface were generally 1° lower than the water temperatures while those 15 cm within the substrate were only 3–4° lower than the water temperatures. Highest substrate temperature at 15 cm depth was 22.6°C.

Except for the ambient channel, weekly diel measurements of total alkalinity within each pool were relatively constant (Fig. 5). Total alkalinity in the ambient channel ranged from 108–118 mg l^{-1} in late May to 146–158 mg l^{-1} in late August. Total alkalinity in the pH 6 channel ranged from 50–82 mg l^{-1} throughout the study. Measurements in the pH 5 channel ranged from 4 to 18 mg l^{-1} except in late May and early June when readings 2 cm above the bottom were 2–6 times higher than at 10 and 30 cm. Concurrent measurements of pH ranges at the same three depths were 7.6–8.5 in the ambient channel, 5.9–6.6 in the pH 6 channel and 4.7–6.4 in the pH 5 channel. The pH readings at the different pool depths usually ranged from 7.9–8.5, 6.2–6.5 and 5.0–5.7 in the ambient, pH 6 and pH 5 channels, respectively.

Total acidity and conductivity varied with the level of acid dosing. Total acidity ranged from 2–6, 42–72 and 64–180 mg l^{-1} in the ambient, pH 6 and pH 5 channels, respectively. Conductivity increased in all channels during the study. In May the ranges were 230–258 μmhos cm^{-1} at 25° (ambient), 269–295 (pH 6) and 282–305 (pH 5) and in August they were 300–327 (ambient, 316–360 (pH 6) and 344–378 (pH 5). Similar levels of total hardness were found in all

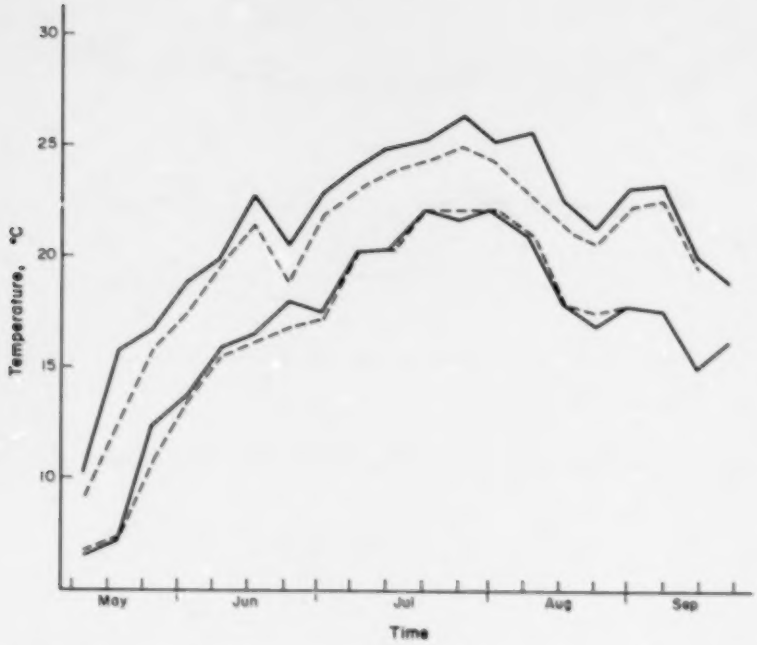

Fig. 4. Weekly maximum and minimum water (solid lines) and substrate (dashed lines) temperatures in the pools.

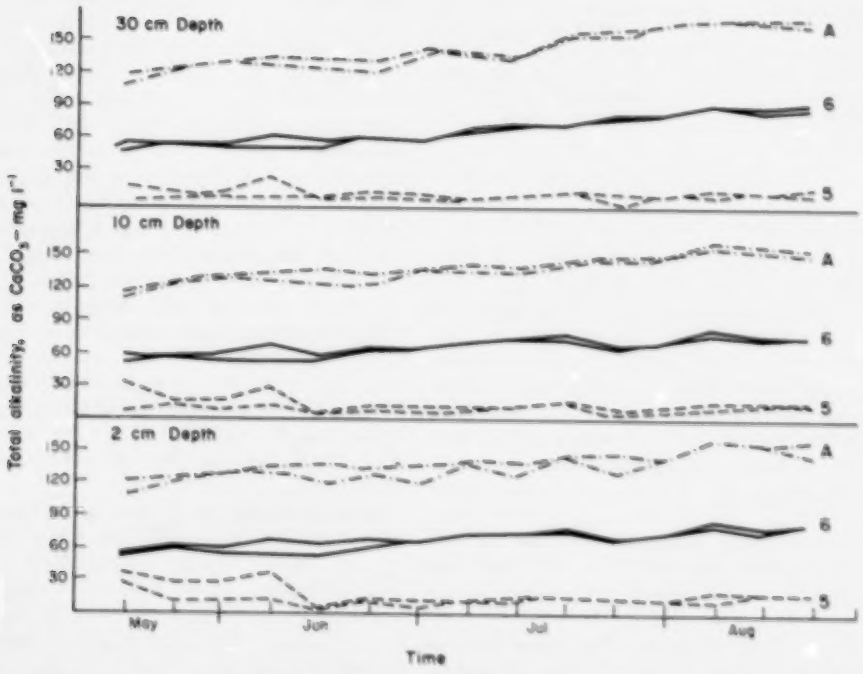

Fig. 5. Weekly maximum and minimum total alkalinity in the pools. A = ambient channel; 6 = pH 6 channel; 5 = pH 5 channel.

three channels and they increased from 114–126 mg l^{-1} in May to 160–167 mg l^{-1} in August.

Little difference was observed between the three channels in the concentrations of dissolved oxygen, nutrients and carbon. Diel variation in dissolved oxygen at the various pool depths was generally 6–10 mg l^{-1}. However, lower readings were found 2 cm above the bottom in the ambient channel at sunrise in late May (4.4 mg l^{-1}) and mid-June (3.6 mg l^{-1}). Nitrogen levels varied as follows: total Kjeldahl 0.8–7.1 mg l^{-1}; ammonia <0.1–0.9 mg l^{-1}; nitrite <0.1–0.2 mg l^{-1} and nitrate <0.1–0.2 mg l^{-1}. Ranges of the other chemical determinations were as follows: total phosphorus <0.1–0.3 mg l^{-1}, total organic carbon 8–14 mg l^{-1} and chemical oxygen demand 23–46 mg l^{-1}.

Monthly metal analyses in the acid dosing tank showed five metals at maximum concentrations of 1–20 mg l^{-1} (Table 1). Ten of 12 elements in the acid tank exceeded concentrations in the diluent river water. Despite high levels in the acid storage tanks, no sustained elevations in metal concentrations were generally noted in the acidified pools. However, in the May samples concentrations of mercury and zinc were 2–3 times higher in the pH 5 channel and mercury was 2 times higher in June, 6 times higher in July and 4 times higher in August in the pH 5 channel than in the ambient channel. The amount of mercury contributed to the channels by the acid was generally 0.1 μg l^{-1} and the amount of zinc added was 0.1–0.2 μg l^{-1}.

Benthic macroinvertebrates

The macroinvertebrate communities in all three channels were dominated by amphipods (*Hyalella azteca* and *Crangonyx* sp. *pseudogracilis* group) and isopods (*Asellus r. racovitzai*) which comprised 45 and 40% respectively, of the total numbers of macroinvertebrates found. Other abundant forms were chironomids (6.1%), planarian flatworms (3.7%), physid snails (2.6%), leeches (0.6%) and damselflies (0.5%). Macroinvertebrates found only periodically but individually totaling >0.01% of the density were, in order of decreasing abundance: caddisflies, sphaeriid clams, mayflies, ceratopogonids, dytiscid beetles, planorbid snails, corixids, stoneflies, elmid beetles, tipulids, simuliids and lymnaeid snails.

Changes in community structure occurred in all channels during the study. The benthic community at the start of the study was largely amphipods and chironomids (Table 2). In the riffles after mid-July, isopods were dominant in the pH 5 channel and amphipods in the pH 6 and ambient channel. In the pools after May, isopods were dominant in all three channels. Although chironomids were commonly present in both riffles and pools early in the study, they comprised no more than 5% of the total density after mid-July. Physid snails never comprised more than 18% and were absent in the pH 5 channel after mid-July. Flatworms were more abundant in the riffles after mid-July especially in the ambient and pH 6 channels. Leeches were found principally in the pools and after mid-July occurred more frequently in the pH 5 channel.

Riffle density of macroinvertebrates was higher in the pH 5 and pH 6 channels (20,398 and 14,789 per m^2, respectively) than in the ambient channel (8,414 per m^2) at the start of the study (Fig. 6). By early June the ambient riffles had 40% greater density than the acidified riffles and from mid-June to early August they were 4–14% higher than the pH 6 channel and 50–68% higher than the pH 5 channel. The benthic density in the acidified channels increased sharply during August relative to the ambient and by September the pH 6 riffles were 71% higher and the pH 5 riffles 38% higher than in the ambient channel. Most of the increased density in the pH 6 channel was due to an increased abundance of amphipods, from 16,956 per m^2 on 17 July to 54,002 per m^2 on 31 August. In the pH 5 channel, increased benthic density in August was caused by a rise in the isopod population, from 12,102 per m^2 on 31 July to 37,346 per m^2 on 6 September.

Benthic densities in the pools fluctuated more than in the riffles (Fig. 6). Preacidification density in the ambient channel (3525 per m^2) was about twice as high as the pH 5 (1777 per m^2) channel and about 30% higher than the pH 6 channel (2399 per m^2). High abundance of chironomids contributed to density peaks in the pH 5 and ambient channels in late May (10,280 per m^2) and early June (12,724 per m^2), respectively. Another density peak in the pH 5 channel occurred in early June (9421 per m^2) and in the ambient channel in late July (6577 per m^2). Benthic density in the pH 6 channel pool increased steadily from mid-May (1007 per m^2) to mid-July (12,517 per m^2) and it exceeded the other two channels from then until the end of the study.

Significant ($P < 0.05$) differences in densities across the three channels were found for five taxa in the riffles and two taxa in the pools during the acidification period. The differences were detected by both the Kruskal–Wallace and analysis of variance tests based on six samples from the riffles and three samples from the pools in each channel within each sampling period. Planarian flatworms were at a higher density in the ambient channel riffles and leeches were more abundant in the pH 5 channel riffles during the entire acidification period. Amphipods in the pH 6 channel riffles and isopods in the pH 5 channel riffles had a greater abundance in the August and September surveys. The density of *Physa gyrina* was greater in the ambient riffles after early July. In the pools, leeches (*Erpobdella*) were more abundant in the pH 5 channel from late May until the end of the study. Isopods were more dense in the pH 6 channel pool except in late May.

Macroinvertebrate diversity was relatively low in the channels. Diversity indices at the beginning of the study were 1.8, 0.9 and 1.0 in the ambient, pH 6 and

Table 2. Per cent occurrence of common macroinvertebrates

Taxon	1 May (Preacid)			15 May			22 May			5 June			19 June			3 July			17 July			31 July			14 Aug.			31 Aug.			6 Sept. (Postacid)			17 Sept. (Postacid)		
	A	6	5	A	6	5	A	6	5	A	6	5	A	6	5	A	6	5	A	6	5	A	6	5	A	6	5	A	6	5	A	6	5	A	6	5
Riffles																																				
Flatworms (*Dugesia*)	—	+	—†	2	1	1	+	3	1	1	+	+	2	2	+	2	1	+	5	19	3	7	3	2	12	9	1	11	8	+	4	6	+	5	19	3
Leeches (*Erpobdella*)	2	1	+	1	1	2	+	+	2	+	+	1	+	+	+	+	+	1	+	—	+	+	+	1	+	+	+	+	+	+	+	+	+	+	—	+
Isopods (*Asellus*)	12	1	4	2	1	4	64	26	19	61	56	40	59	56	47	46	48	56	27	19	65	30	35	62	23	21	59	26	16	56	42	31	55	27	19	65
Amphipods (*Hyalella, Crangonyx*)	39	86	82	37	79	74	4	58	68	16	25	42	35	36	49	46	34	41	49	58	28	52	56	33	55	63	37	51	73	41	42	59	42	49	58	28
Damselflies (*Enallagma, Ischnura*)	—	+	+	+	1	1	—	1	+	+	+	+	+	–	+	+	+	+	1	+	1	+	+	+	2	+	+	2	+	1	2	+	1	1	+	1
Chironomids	44	9	12	55	14	15	30	9	7	19	16	14	2	3	3	1	13	1	3	1	1	+	1	1	2	2	1	3	+	+	1	+	+	3	1	1
Snails (*Physa*)	—	1	+	+	1	1	—	2	+	+	1	+	+	+	+	4	2	+	9	2	+	10	4	—	3	4	—	6	2	—	7	2	—	9	2	+
Pools																																				
Flatworms (*Dugesia*)	—	—	—	1	—	1	+	—	+	—	+	—	—	—	1	—	—	+	—	—	—	—	—	—	3	—	—	—	—	—	—	—	—			
Leeches (*Erpobdella*)	3	9	8	3	16	11	+	8	5	+	3	8	18	2	12	—	2	3	1	1	17	2	3	24	—	4	28	2	5	42	2	1	38			
Isopods (*Asellus*)	1	8	3	—	—	5	1	19	4	35	64	27	38	77	77	71	72	95	63	71	21	68	85	62	50	56	66	42	91	37	35	93	49			
Amphipods (*Hyalella, Crangonyx*)	9	48	22	—	10	12	2	16	1	8	5	23	9	10	2	5	12	1	23	16	51	19	5	9	7	36	2	1	3	20	+	5	9			
Damselflies (*Enallagma, Ischnura*)	1	2	10	—	3	—	1	—	+	+	2	6	—	+	3	—	+	—	—	—	—	1	+	—	3	—	1	1	—	—	—	1	+	3		
Chironomids	83	31	34	91	69	44	85	57	84	53	25	25	31	6	1	22	11	1	4	2	4	1	—	1	5	2	1	4	+	—	5	+	1			
Snails (*Physa*)	—	—	18	—	—	15	—	—	1	+	+	9	—	3	3	—	1	+	1	5	—	1	3	—	16	—	—	3	—	—	2	—	—			

*A refers to ambient channel; 6 to pH 6 channel; 5 to pH 5 channel.
† + refers to taxa < 1%; — to taxa not found.

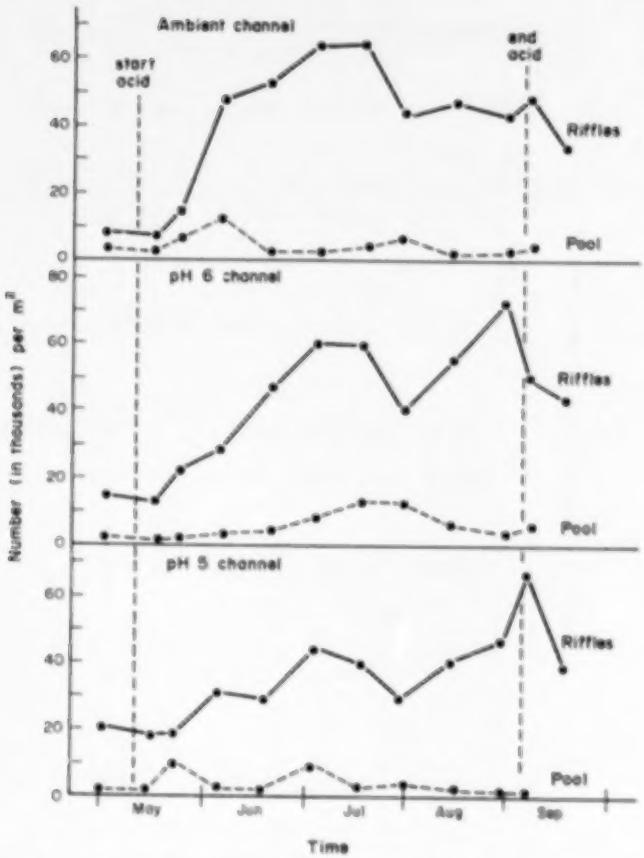

Fig. 6. Benthic macroinvertebrate densities in the riffles and pools.

pH 5 riffles, respectively. Riffle indices by early June were identical in all three channels (1.7). At the end of the acidification period, the ambient riffle index was 2.0, and in the pH 6 and pH 5 channels it was 1.4 and 1.2, respectively. Diversity in the pools during the acidification period increased from 1.3 to 1.6 in the ambient channel, decreased from 1.9 to 0.5 in the pH 6 channel, and decreased from 2.7 to 1.5 in the pH 5 channel.

Few differences were observed in growth and reproductive activity of amphipods, isopods and physid snails. Gravid *Crangonyx* sp. females were collected at every sampling period and similar size frequencies were observed in all three channels. *Hyalella azteca* also showed similar size frequencies in the three channels. After May, a sharp drop in *H. azteca* abundance occurred in the pH 5 channel and fewer than 20 individuals were collected in the remaining sampling periods. Newborn *H. azteca* were observed in all three channels on 5 May and in the ambient and pH 5 channels on 14 August. Gravid *Asellus r. racovitzai* females were collected throughout the study. Newly released young were first observed in the 15 May samples from the ambient and pH 6 channel and the 22 May samples from the pH 5 channel. First egg masses of *Physa gyrina* were found in all three channels at the end of May with newly hatched snails first appearing in the 27 June samples. Less than 30 individuals were collected in the pH 5 channel from late May through July and none were collected thereafter. No differences were observed in the snail growth in the ambient and pH 6 channels throughout the acidification period. Another generation of newly hatched snails was found in the September samples from the ambient but not from the pH 6 channel.

The drift densities of the common macroinvertebrates varied between channels and between the four monthly diel samples within the same channel (Fig. 7). Total drift was higher at night than during the daytime. Amphipods comprised 70% of the total drift numbers. Other taxa commonly present in the drift were, in order of decreasing abundance: damselflies, physid snails, corixids, leeches, mayflies (10 species), chironomids, isopods and dytiscid beetles. Total nocturnal (1900–0500 CST) density in the May survey was 3.5 times higher in the pH 5 channel than in the other two channels; amphipods were 5 times and leeches 15 times more abundant in the pH 5 channel.

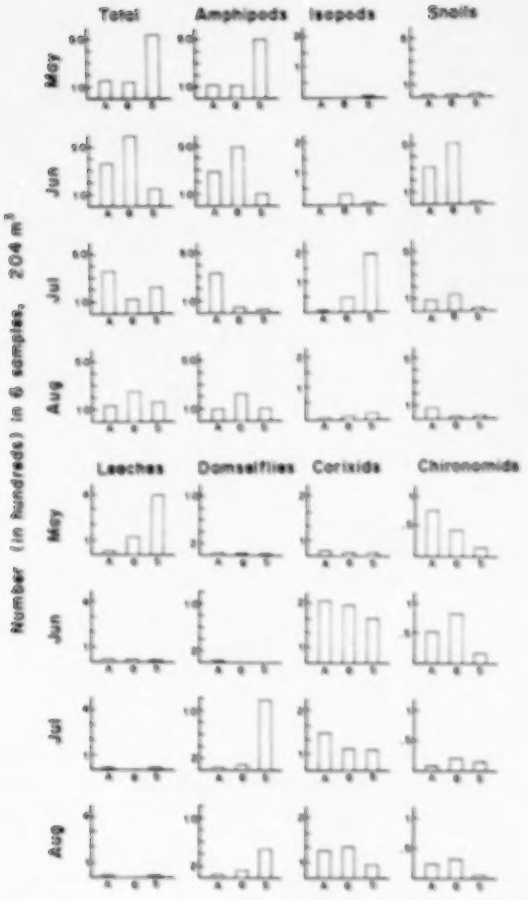

Fig. 7. Nocturnal drift (1900–0500 CST) of total macroinvertebrate and seven representative taxa during four sampling periods. A = ambient channel; 6 = pH 6 channel; 5 = pH 5 channel.

Mayflies, corixids and newly hatched physid snails were abundant by June in the drift, and total density was highest in the pH 6 channel followed by the ambient and pH 5 channel. July drift densities in the pH 5 and pH 6 channels were 61 and 33%, respectively, of the ambient channel. Isopods and damselflies were abundant in the pH 5 channel. In August, total density was highest in the pH 6 channel followed by the pH 5 and ambient channel.

Insect emergence

Chironomids comprised 92–94% of the total number of insects emerging from the three channels (Table 3); the subfamilies Chironominae, Orthocladinae, Tanypodinae and the tribe Tanytarsini comprised 20, 16, 6 and 58%, respectively of the total. Sex ratios (male to female) of the chironomids were approximately equal (50 ± 10%). Trichopterans, the second most common order, accounted for < 1–8% of the total. The other emergent insects, in order of decreasing abundance were Diptera (other than chironomids), Lepidoptera, Zygoptera and Ephemeroptera.

The numbers of different insect taxa emerging from the ambient, pH 6 and pH 5 channels were 30, 28 and 18, respectively, (Table 3). Chironomid taxa numbers were 22 in the ambient channel, 18 in the pH 6 channel and 11 in the pH 5 channel. Different, but unidentified, species in the same genus of the Chironomidae are designated by letters. Damselflies and the aquatic lepidopteran *Synclita* emerged only from the acidified channels. Only one caddisfly species and no mayflies emerged from the pH 5 channel. *Tanytarsus* spp were the most abundant chironomids in the ambient channel; *Micropsectra* sp. C was the most numerous in the pH 6 and pH 5 channels.

Most of the insect taxa emerged in approximately equal numbers from the riffles and pools. However, three species were collected mainly in the riffle traps. More than 97% of the *Chesmatopsyche* and *Nemicladius* emerged from the riffles; all of the *Oecetis immobilis* emerged from the riffles.

Table 3. Total insect numbers emerging and per cent occurrence of major groups and identified taxa within each group

Taxon	Ambient channel			pH 6 channel			pH 5 Channel		
	Total number	Per cent by group	Per cent within group	Total number	Per cent by group	Per cent within group	Total number	Per cent by group	Per cent within group
Chironomidae	1806	92		993	92		423	94	
Apedilum sp.			+*			—*			—
Chironomus atrella (Townes)			+			+			—
Clinotanypus pinguis (Lowe)			+			—			+
Conchapelopia sp.			—			+			—
Cornynoneura sp.			+			—			—
Cricotopus sp.			+			6			—
Dicrotendipes fumidus (Johannsen)			+			+			—
Dicrotendipes modestus (Say)			+			—			—
Endochironomus nigricans (Johannsen)			+			+			—
Micropsectra sp. A			6			17			25
Micropsectra sp. B			+			+			19
Micropsectra sp. C			16			32			33
Nanocladius rectinervis (Kieffer)			16			7			+
Orthocladius sp.			+			+			+
Parachironomus sp.			+			+			+
Paratendipes albimanus (Mg.)			11			7			+
Polyedlium illinoenses (Malloch)			+			+			+
Polypedilum nebeculosum (Mg.)			—			+			—
Procladius sp.			+			+			+
Pseudochironomus richardsoni (Malloch)			+			+			—
Tanypus punctipennis (Meigen)			+			—			—
Tanytarsus spp			24			11			+
Xenochirnomus senolabis (Kieffer)			+			+			—
Zavrelimyia thryptica (Sublette)			+			—			—
Unidentified			+			+			+
Ephydridae	2	+	+	7	+	+	3	+	+
Simuliidae	4	+	+	1	+	+	2	+	+
Ephemeroptera	5	+		3	+		0	—	
Caenis			+			+			—
Callibaetis			+			+			—
Lepidoptera	0	—		5	+		11	+	
Synclita			—			+			+
Trichoptera	153	8		57	5		6	+	
Cheumatopsyche sp.			55			84			+
Hydroptila sp.			+			—			—
Oecetis immobilis Hagen			39			16			—
Zygoptera	0	—		12	+		3	+	
Enallagma sp.			—			+			+
Ischnura			—			+			+
Total insects	1970			1078			448		

* + refers to taxa <5.0%, — to taxa not found.

Emergence of insects from the three channels was similar during the first 5 weeks of the study. After 2 weeks of acid dosing, a marked decline in numbers occurred in the pH 5 channel (Fig. 8). Of total insect numbers emerging throughout the study, 60% were from the ambient channel, 31% from the pH 6 channel and 9% from the pH 5 channel. The average number of insects collected each week were 89, 49 and 20 in the ambient, pH 6 and pH 5 channel, respectively.

The number and size of emergence peaks were larger in the ambient channel than in the acidified channels (Fig. 8). Chironomids were the only group emerging in large numbers (>20) until late June. The large emergence in early June from the ambient and pH 6 channels was composed primarily of *Micropsectra* sp. C; there was not a similar emergence from the pH 5 channel. An emergence peak from the ambient channel in early July consisted of the chironomids, *Paratendipes albimanus*, and *Tanytarsus* spp and the caddisfly, *Oecetis immobilis*; there was not a peak in the acidified channels. The emergence peak in late August in the pH 6 channel was primarily *Micropsectra* sp. C. Higher insect emergence from the ambient channel during the last 5 weeks of the study was due mainly to abundance of *Cheumatopsyche* and *Nanocladius* spp. The only species that had emergence limited to a 2–3 week period were *Micropsectra* spp A and B, and they emerged in early May from all three channels.

The emergence traps remained in the channels for 7 days after termination of the acid dosing. During this period the total numbers of insects emerging increased in the pH 5 channel and decreased in the ambient and pH 6 channels.

Fish

Growth of free-roaming fathead minnows prior to spawning was similar in all three channels. Mean weights of the fish in all channels increased from 0.6 g on 17 April to 0.9 g on 23 May. By 6 June the fish had doubled in weight and by 13 June, when male secondary sexual characteristics first appeared, they had increased to 1.4, 1.6 and 1.4 g in the ambient, pH 6 and pH 5 channels, respectively.

Over 99% of all spawning activity occurred in the ambient and pH 6 channels (Table 4). Spawning was first observed in the ambient channel during the week of 18–22 June and in the pH 6 and pH 5 channels 1 and 2 weeks later, respectively. The duration of the spawning period, as determined by examining the substrates, was appproximately twice as long in the pH 6 channel (25 June–17 August) as in the ambient channel (25 June–20 July). Egg counts as determined by water volume displacement, were approx. 73% higher in the pH 6 channel than in the ambient channel. However, total eggs estimated, by visual examination of spawning substrates, were 24% higher in the ambient than in the pH 6 channel. Eggs that developed to the eyed-embryo stage were estimated to range from 18 to 66% in the ambient channel and 33–100% in the pH 6 channel. Mean numbers of eggs per spawning substrate in the ambient channel were approx. 62% higher than in the pH 6 channel. Ambient channel females utilized 44 spawning substrates as compared to 61 in the pH 6 channel. Eggs

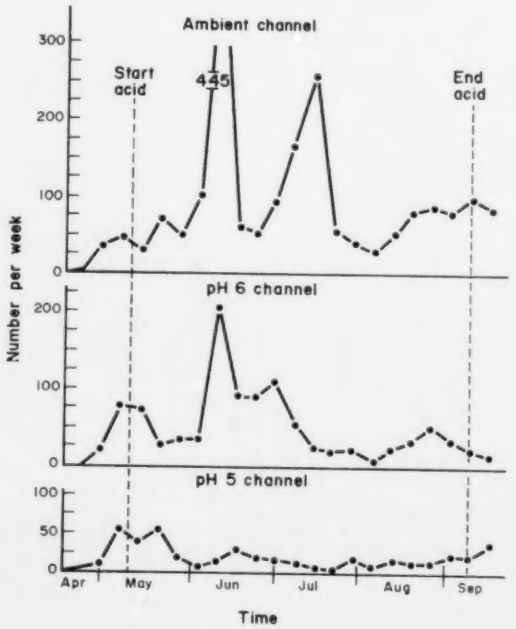

Fig. 8. Weekly catches of emerging insects.

Table 4. Spawning of fathead minnows

Date*	Eggs counted and removed: Ambient channel Eggs	Ambient channel Spawning sites	pH 6 channel Eggs	pH 6 channel Spawning sites	pH 5 channel Eggs	pH 5 channel Spawning sites
27 June	6400	6	1350	2	0	—
2 July	2030	3	5250	9	23	1
9 July	0	—	3570	7	0	—
16 July	0	—	3050	4	0	—
23 July	0	—	1360	3	0	—
Total	8430	9	14580	25	23	—
Mean eggs/ spawning site		940		580		23

Date*	Number of eggs estimated: Ambient channel Eggs	Ambient channel Per cent eyed embryos	Ambient channel Spawning sites	pH 6 channel Eggs	pH 6 channel Per cent eyed embryos	pH 6 channel Spawning sites	pH 5 channel Eggs	pH 5 channel Per cent eyed embryos	pH 5 channel Spawning sites
18 June	1900	20	5	0	—	—	0	—	—
27 June	28000	40	16	7500	40	7	0	—	—
2 July	7300	60	12	7400	60	9	50	0	1
9 July	3300	40	9	6200	40	12	60	0	1
16 July	300	70	2	2300	30	5	0	—	—
23 July	0	—	—	3300	40	7	0	—	—
30 July	0	—	—	4400	50	9	0	—	—
6 August	0	—	—	1900	50	8	0	—	—
13 August	0	—	—	100	100	4	0	—	—
Total	41000		44	33100		61	110		2
Mean eggs/ spawning site		900			500			60	

*Monday of week sampled.

were observed in the pH 5 channel from 2 to 13 July. However, none of the eggs in the pH 5 channel developed to the eyed-embryo stage. Spawning was concentrated on the four upstream substrate columns in all channels and occurred more frequently on the substrates at mid-depth.

Distributional studies of free-roaming fish showed their living space centered principally in the pools where approx. 90% of the fish were captured. In the ambient and pH 5 channels 64 and 54%, respectively, of the fish were trapped in the upstream section of the pool but 51% were trapped in the downstream section in the pH 6 channel. Fish catches at the surface to mid-water depth were 71% higher than in deeper water. Most of the fish captures in the riffles occurred during the day and about 80% were trapped in the upper riffles.

Nearly equivalent nocturnal drift densities of newly-hatched larvae (< 1.0 cm long) were recorded at the downstream (export) ends of the study sections in the ambient and pH 6 channels (822 and 769, respectively). A total of 59 exported fish larvae were collected in the pH 5 channel. Largest quantities of export fish drift occurred during the last week of June in the ambient channel (562 fish) and the second and third weeks of July in the pH 6 (399 fish) and pH 5 (56 fish) channels, respectively. The peak drift period was from 2100 to 0100 CST. Few fish larvae (six and one, respectively) were caught in the import drift nets at the upstream end of the first riffles in the ambient and pH 5 channels and none were collected in the pH 6 channel. Apparently some spawning occurred from fish that escaped upstream from the experimental sections.

Approximately 99.5% of all the juvenile minnows (1.9–5.5 cm long) collected from the pools downstream from the study sections and at the discharge sites were from the ambient channel (2924 fish from pools, 76 fish at discharge). Only 14 and 1 fish were collected at the downstream locations in the pH 6 and pH 5 channels, respectively.

Final numbers and size frequencies of fish collected from the three channels at the end of the study are shown in Fig. 9. Approximately 3 times more fish were recovered from the ambient channel than the other two channels. The fish length ranged from 2.0 to 8.5 cm in the ambient channel, 3.5–8.5 cm in the pH 6 channel and 6.0–9.0 cm in the pH 5 channel. The fewest adult fish (> 6.0 cm long) were recovered from the pH 6 channel. Numbers of fish greater than 6.0 cm long were 102, 63 and 129 in the ambient, pH 6 and pH 5 channels, respectively.

Several other species of fish were found in the study sections of the channels. Fishes recovered at the end of the study were johnny darters, bluegills, suckers and bullheads. Total numbers present in the ambient, pH 6 and pH 5 channels were 19, 4 and 5 respectively.

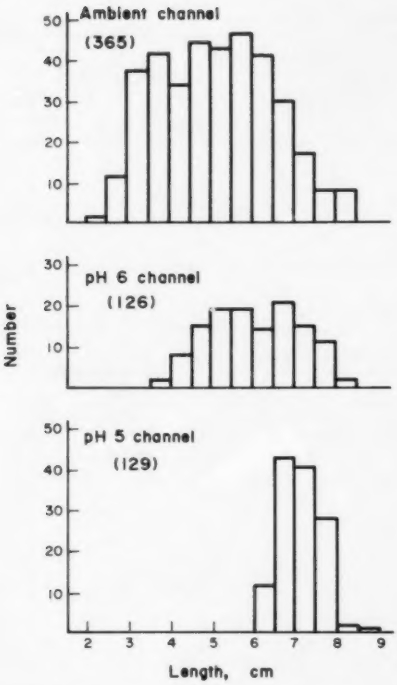

Fig. 9. Final fathead minnow size distribution. Number of fish measured in parenthesis.

The average size of the imported fish was 5.5 cm; the largest, a redhorse sucker, measured 10.5 cm.

Survival of caged fish was lowest at pH 5, but there was little difference in their growth in the three channels (Table 5). In all three channels survival was 100% through the first week of May. However, survival at pH 5 on 8 June was less than 50% compared to more than 85% in the other two channels. Fish survival in the ambient and pH 6 channel cages on 16 August was 75–95% compared to 25–30% in the pH 5 channel. Final mean weight of caged fish in the ambient channel was slightly higher than in the other two channels.

DISCUSSION

Acidification of the outdoor experimental channels changed several chemical properties of the river water. The most marked changes with decreasing pH were a successive lowering of total alkalinity and a concomitant increase in total acidity. Glass & Loucks (1980) concluded that low total alkalinity was an important feature of a lake's vulnerability to acidification. The alkalinity values in the pH 5 channel (4–18 mg l^{-1}) were within the range where fishery damage in lakes can occur. Hall *et al.* (1980) found significant mobilization of aluminum and calcium (9 and 181%, respectively) in their stream acidification study. Schindler *et al.* (1980) also demonstrated the release of four metals into tubes inserted in the bottom sediment of an acidified lake. Zinc showed the greatest increase into the overlaying lake water from $< 1\ \mu g\ l^{-1}$ in the control tubes (pH 6.7–6.8) to $> 300\ \mu g\ l^{-1}$ in tubes at pH 5. In our study, little evidence of metal mobilization was found within the experimental channel sections except possibly zinc and mercury. In May the zinc concentration in the pH 5 channel was nearly 3 times higher than in the ambient channel and during May, June, July and August the mercury concentrations in the acidified channels were 2–6 times higher than in the ambient channel. The maximum amount of mercury added to the channels by the acid source was calculated to be approx. 0.1 $\mu g\ l^{-1}$ in June when the measured metal concentrations were 0.3 $\mu g\ l^{-1}$ in the ambient channel and 0.6 $\mu g\ l^{-1}$ in the pH 5 channel. It appears, therefore, that acidification might have caused mercury mobilization up to 0.2 $\mu g\ l^{-1}$ in the pH 5 channel. Although the biological effects demonstrated in our study can be primarily attributed to reduced pH, elevated mercury levels cannot be entirely excluded as a possible mitigating factor.

Reduction in invertebrate diversity and abundance have been demonstrated in other acidified aquatic ecosystems. Herricks & Cairns (1974) reported a 42% reduction in invertebrate benthic density and a 30% diversity decrease in a brief acidification experiment. Similarly, Hall *et al.* (1980) found a lower diversity

Table 5. Survival and growth of caged fathead minnows

Date	Ambient channel Cage A	Ambient channel Cage B	pH 6 channel Cage A	pH 6 channel Cage B	pH 5 channel Cage A	pH 5 channel Cage B
			Survival (%)			
27 April	100	100	100	100	100	100
9 May	100	100	100	100	100	100
8 June	90	95	95	90	55	25
7 June	85	90	95	85	50	25
16 August	80	85	95	75	30	25
			Mean weight(g)			
27 April	0.7	0.7	0.7	0.7	0.6	0.7
16 August	3.1	2.8	2.3	NM*	2.4	3.0

*Not measured.

and a 75% density reduction in a stream section acidified for 5 months. Benthic densities were lower in the pH 5 and pH 6 channels during most of the first 3 months of the study but densities at the end of the acidification period were higher in the treated channels because of increased abundance of tolerant species. Final diversities were 30–40% lower in the acidified channels than in the ambient channel.

The acidification stimulated macroinvertebrate drift activity. Total nocturnal drift early in the study was 3.5 times higher in the pH 5 channel when benthic densities were similar in all three channels. Amphipods and leeches drifted at rates 5–15 times higher in the pH 5 channel than in the ambient channel. In July isopod drift densities were 12 and 49 times higher than the ambient in the pH 6 and pH 5 channels, respectively, yet benthic densities were only 92 and 49% higher in the two acidified channels. Hall *et al.* (1980) found total drift increased 13 times in the first week of acidification but the effect was not prolonged. They categorized their drifting invertebrates into three groups: (1) those that did not respond to increased acidity; (2) those drifting only after acidification; and (3) those with increased drift after acidification. Most of their genera were in the latter category. We conclude that of the common macroinvertebrates in our drift samples, mayflies, corixids, and chironomids were in the first category; isopods and leeches in the second; and amphipods, physid snails and damselflies in the third category.

Chironomids, the most abundant insect emerging from the channels, are normally limited to waters with pH higher than 5.5 although a few species have been recorded from waters as low as pH 3 (Roback, 1974). Wojcik & Butler (1977) observed an emergence of chironomids from a stream receiving acid mine drainage with a pH of 3.4. Under conditions of acid stress, emergence of aquatic insects appears to be the critical stage of the life cycle (Bell, 1971). Hall *et al.* (1980) observed a 37% reduction in emergence of insects from their acidified stream. Bell (1970) reported that *Tanytarsus dissimilis* was unable to emerge at pH 5. He also concluded that this chironomid was unsuccessful in emerging due to the inability of the adult to escape from the pupal case. We collected a species of *Tanytarsus* in percentages of 3, 22 and 75% from the pH 5, pH 6 and ambient channels, respectively. *Micropsectra* spp A and C were the midges most tolerant of acid stress; they emerged from all three channels throughout our study. Both total abundance and number of adult insect species emerging from the channels decreased with increasing acid stress.

Relatively low numbers of caddisflies, damselflies and lepidopterans emerged from the acidified channels. Bell & Nebeker (1969) found caddisflies to be the most tolerant of nine insect species tested. Progressively lower numbers of caddisflies emerged from the ambient, pH 6 and pH 5 channels and only *Cheumatopsyche* emerged from the pH 5 channel. Of the few damselflies that emerged, most were from the pH 6 channel. The aquatic lepidopteran *Synclita* normally occurs in the range of pH 5.6–6.6 (Roback, 1974). *Synclita* emerged from the acidified channels after 4 months of acid dosing.

The sensitivity of macroinvertebrates can be defined from this field study and laboratory data. The planarian *Dugesia* occurred in all three experimental channels during the acidification period but significantly higher populations occurred in the ambient channel. Planarian flatworms were not reported in acid conditions in a survey of Wisconsin ponds (Tucker, 1958). The leech *Erpobdella punctata*, which shows wide pH tolerance limits (from 5.0 to 8.0) in natural waters (Sawyer, 1974), was significantly more abundant in the pH 5 channel during acidification. Snails characteristically have low tolerance to acid stress. Okland (1969) found the following in a survey of acidified Swedish lakes: pH <5.2 no snails, from 5.2 to 5.8 snails rare, and at pH values of 5.8–6.6 snails occurred less frequently than in neutral or alkaline waters. Harrison & Agnew (1962) reported that no snails occurred in acid streams below pH 6. *Physa gyrina* has been reported living in lakes in New York at pH 6.7–8.3 (Harman & Berg, 1971). Snails were eliminated from the pH 5 channel after 10 weeks of acidification and were at significantly lower density in the pH 6 channel during the same period. Okland (1979) reported that the isopod *Asellus aquaticus* tolerates pH values down to about 5.0. *A. r. racovitzai* produced a large population at the lowest pH in our experiment. Of the two amphipod species in our study, *H. azteca* was more sensitive to acid stress and nearly disappeared from the pH 5 channel by June. Large populations of *Crangonyx* were present in all channels throughout the study, although they were less abundant in the lowest pH. Damselfly nymphs were present in the benthic and drift samples from all three channels throughout this study. The damselfly genera present in our channels have been found in natural water at the following pH ranges: *Enallagma* pH 5.6–8.4, *Ischnura* pH 6.8–8.5 (Roback, 1974). Kimmel and Hales (1973) reported a low TLm 96 of pH 2.25 for the damselfly *Boyeria vinosa*. Based on these findings and our results, the leeches, isopods and damselflies were judged to be acid tolerant. Intermediate in sensitivity were flatworms, the amphipod *Crangonyx* and chironomids, although the tolerance of the different midge species probably varies widely. Most sensitive to acidification were the amphipod *H. azteca* and the snail *P. gyrina*.

The principal effects of acidification on the fathead minnow were on reproduction and young fish survival. There was no evidence of an avoidance response by fish in the acidified channels. Spawning intensity, duration of the spawning period and reproductive success were similar in the ambient and pH 6 channels, but little spawning and no eyed-embryos were observed in the pH 5 channel. Numbers of newly-hatched larvae collected from drift were about

equal in the ambient and pH 6 channels suggesting that these pH values did not affect egg hatchability. Mount (1973) exposed fathead minnows to four different pH levels from 4.5 to 6.6 and a control for 13 months. He observed definite reductions in egg production and hatchability at pH 5.9 and slight decreases at pH 6.6, compared to the control. In a study conducted at our field station, McCormick (1980) incubated minnow eggs collected from the ambient and pH 6 channels. Hatching success was significantly lower for eggs incubated at pH 6, a result that is inconsistent with our findings. Reproductive failure at pH 5 may have resulted from inhibition of egg maturation. Failure of white suckers to spawn at low pH has been related to an inability to maintain normal serum calcium levels during the period of ovarian maturation (Beamish *et al.*, 1975). Ruby *et al* (1977) reported significant reductions in the numbers of stage 1, stage 5 and stage 6 oocytes in flagfish exposed to pH 5 and a reduction in stage 6 oocytes exposed to pH 6. His group concluded that reduced egg formation was the result of faulty deposition of protein in developing oocytes. Almost all of the juvenile minnows collected in our study were from the ambient channel, less than 1% were recovered in the acidified channels. Survival of caged fish was about 50% lower in the pH 5 channels. Our findings are similar to those of Craig & Baksi (1977) who reported the following order of sensitivity to acid stress for the flagfish: egg production > fry survival > fry growth > egg fertility. We also confirmed Mount's (1973) conclusion that a pH 5 is clearly unacceptable to fathead minnows.

From our results predictions can be made concerning the consequences of long-term acidification of the channels. The overall result of prolonged acidification on macroinvertebrates would be to further decrease community diversity while increasing the abundance of the more tolerant species. Continued acidification would likely have caused extinction of the fathead minnow populations at both pH 5 and pH 6. Our results suggest no-effect pH values would be in excess of 6.0 in the outdoor channels, and give support to the proposed protection pH recommendations (6.5 or higher) set by the National Academy of Sciences (1973) and the U.S. Environmental Protection Agency (1976).

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